

PREPARATION FOR SEQUENCING

DIFFERENT SAMPLE PREPARATION METHODS FOR DIFFERENT TYPE OF NGS DATA

- DNaseq
- RNASeq
- CHIPSeq
- Metagenomics
- Single Cell

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Next Generation Sequencing

NGS is a emerging technology for DNA and RNA sequencing and variant/mutation detection. This technology combines the advantages of unique sequencing chemistries, different sequencing matrices, and bioinformatics technology. Such a combination allows a massive parallel sequencing of various lengths of DNA or RNA sequences or even whole genome within a relatively short period of time.

Techniques

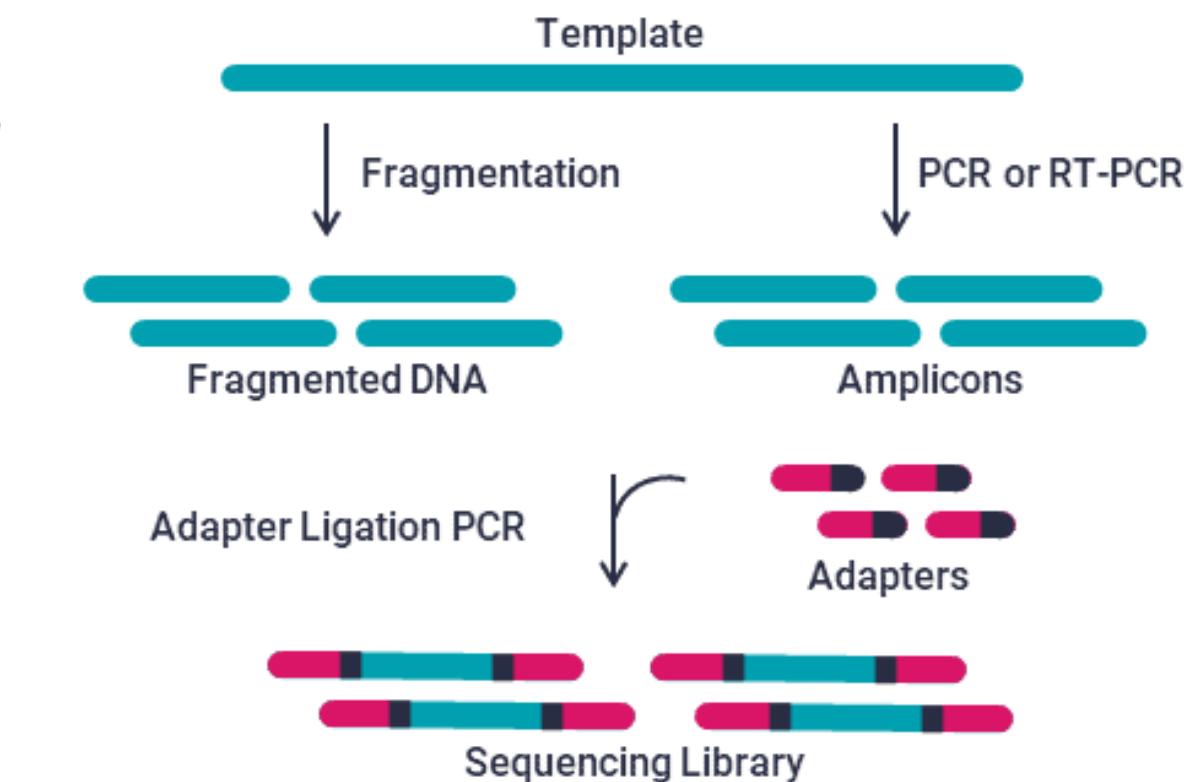
- ILLUMINA SEQUENCING
- ROCHE 454 SEQUENCING
- ION TORRENT SEQUENCING

DNA Sequencing

STEP 1: Extraction



STEP 2: Library Prep



STEP 3: Sequencing



STEP 4: Analysis



>read1
aacgctcgtagttagctct
agctacggatcgctacgg
ctaggtaactcgctatctata
aaaactccgcgtcgatctacg
gcgatcgactcgatctacgc
ggtttgtaccgcatactacg
ccgatctagc



1. Sample Preparation:

- Extract DNA from the sample
- Fragment the DNA into smaller pieces

2. Library Preparation:

- Adapters are ligated to the fragmented DNA
- DNA fragments are amplified via PCR

3. Sequencing:

- DNA fragments are loaded onto the sequencing platform
- Sequencing chemistry is applied to determine the sequence of each fragment

4. Data Analysis:

- Raw sequencing data is generated
- Reads are aligned to a reference genome or assembled de novo

Advantages

- High Throughput
- Speed
- Cost - Effectiveness



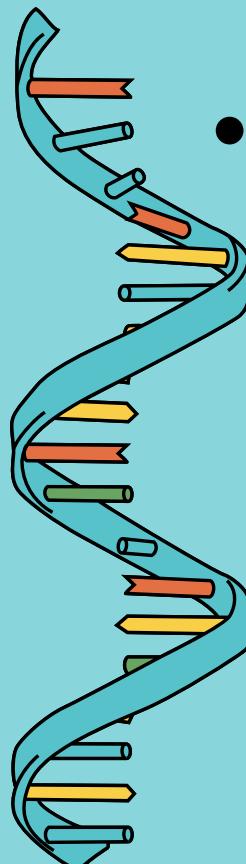
Disadvantages

- Sample preparation requirements
- Data Complexity

RNA-Seq

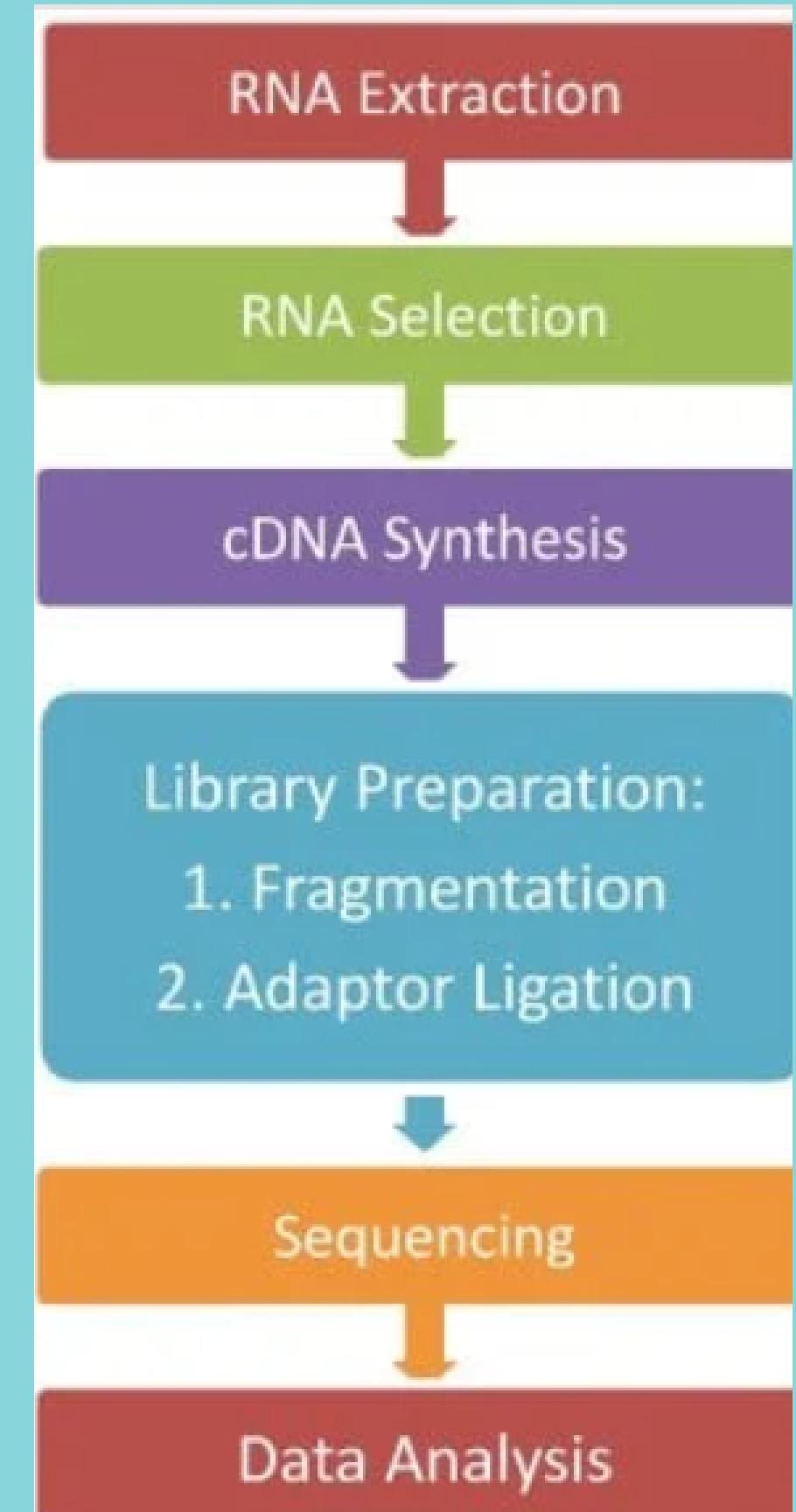
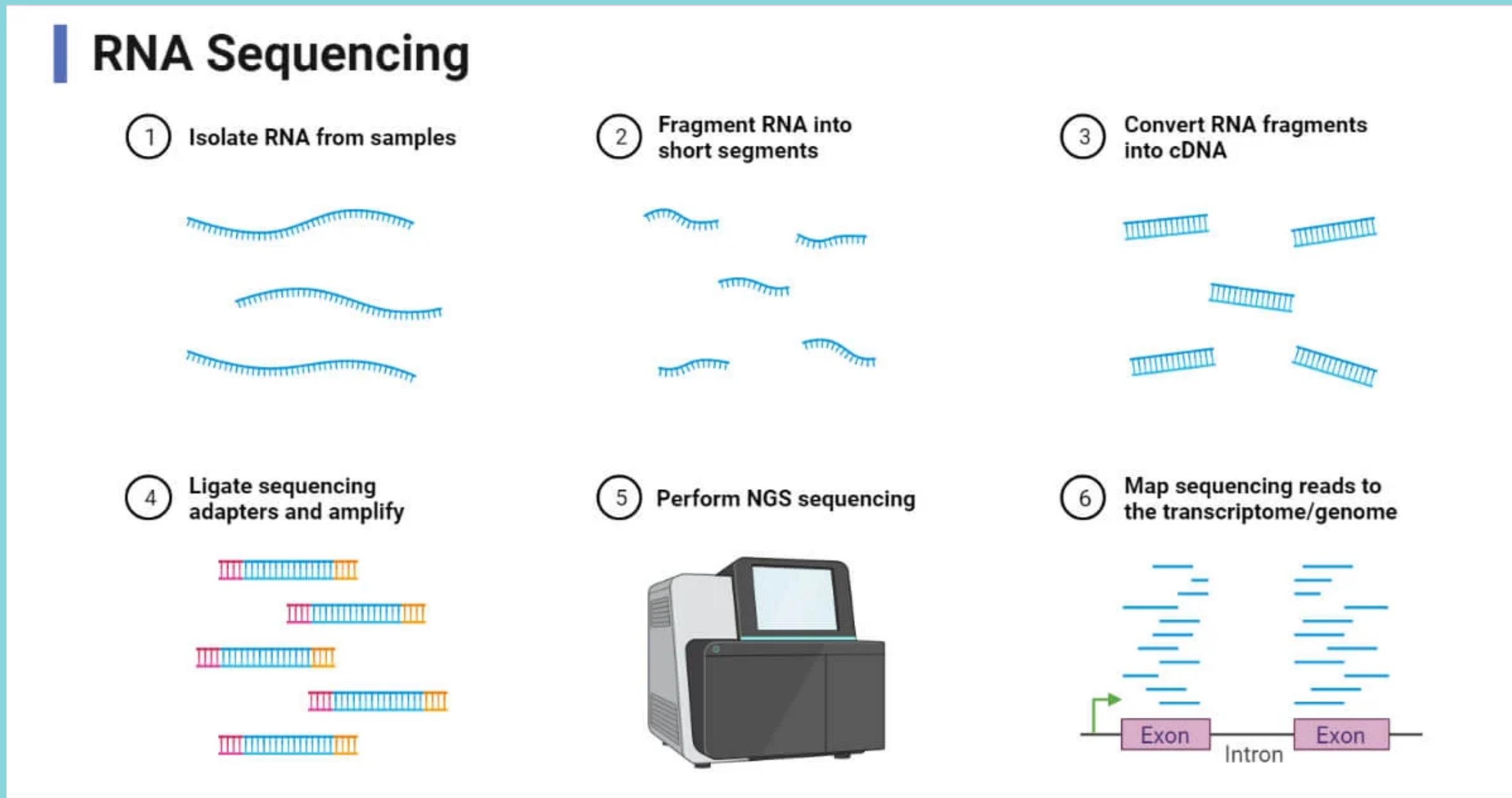
RNA-Sequencing (RNA-Seq) is a powerful technique used to analyze the **transcriptome** of cells or tissues. It provides comprehensive insights into gene expression patterns, alternative splicing, RNA editing, and transcript isoform diversity.

Why to do RNA-Seq analysis?



- **RNA-Seq includes experiments to**
 - Profile abundance of mRNA and other RNAs.
 - Identify "differentially expressed" genes.
 - Identify alternated spliced transcript isoforms.
 - Assemble transcriptome.

RNA-Sequencing Workflow:-



The process of RNA sequencing involves converting RNA molecules into complementary DNA (cDNA) and then sequencing the cDNA fragments. The steps involved in RNA sequencing typically include:

RNA extraction: RNA is isolated from the biological sample of interest. This can be done using various methods, such as using commercial RNA extraction kits or specialized protocols for different sample types.

RNA fragmentation: The extracted RNA is fragmented into smaller pieces. This step is necessary to facilitate the subsequent cDNA synthesis and sequencing.

cDNA synthesis: The fragmented RNA molecules are reverse transcribed into complementary DNA (cDNA) using reverse transcriptase enzymes. This step converts RNA into a stable and amplifiable form for sequencing.

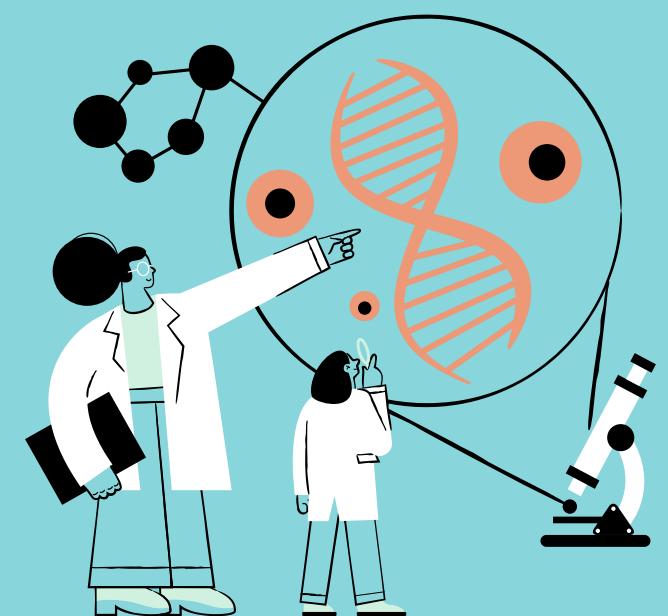
Library preparation: The cDNA fragments are modified to add specific adapters that contain sequences necessary for sequencing, such as primer binding sites. This step prepares the cDNA fragments for sequencing and allows multiple samples to be sequenced together in a single sequencing run.

Sequencing: The cDNA libraries are loaded onto a high-throughput sequencing platform, such as next-generation sequencing (NGS) instruments. The cDNA fragments are sequenced, producing millions of short sequence reads.

Data analysis: The generated sequencing reads are then processed and analyzed using bioinformatics tools and pipelines. This involves aligning the reads to a reference genome or transcriptome, quantifying gene expression levels, identifying alternative splicing events, and detecting novel transcripts or non-coding RNAs.

Application Of RNA Seq:-

- Small RNA profiling
- Characterization of alternative splicing patterns
- System biology
- Single-cell RNA-seq
- Differential gene expression

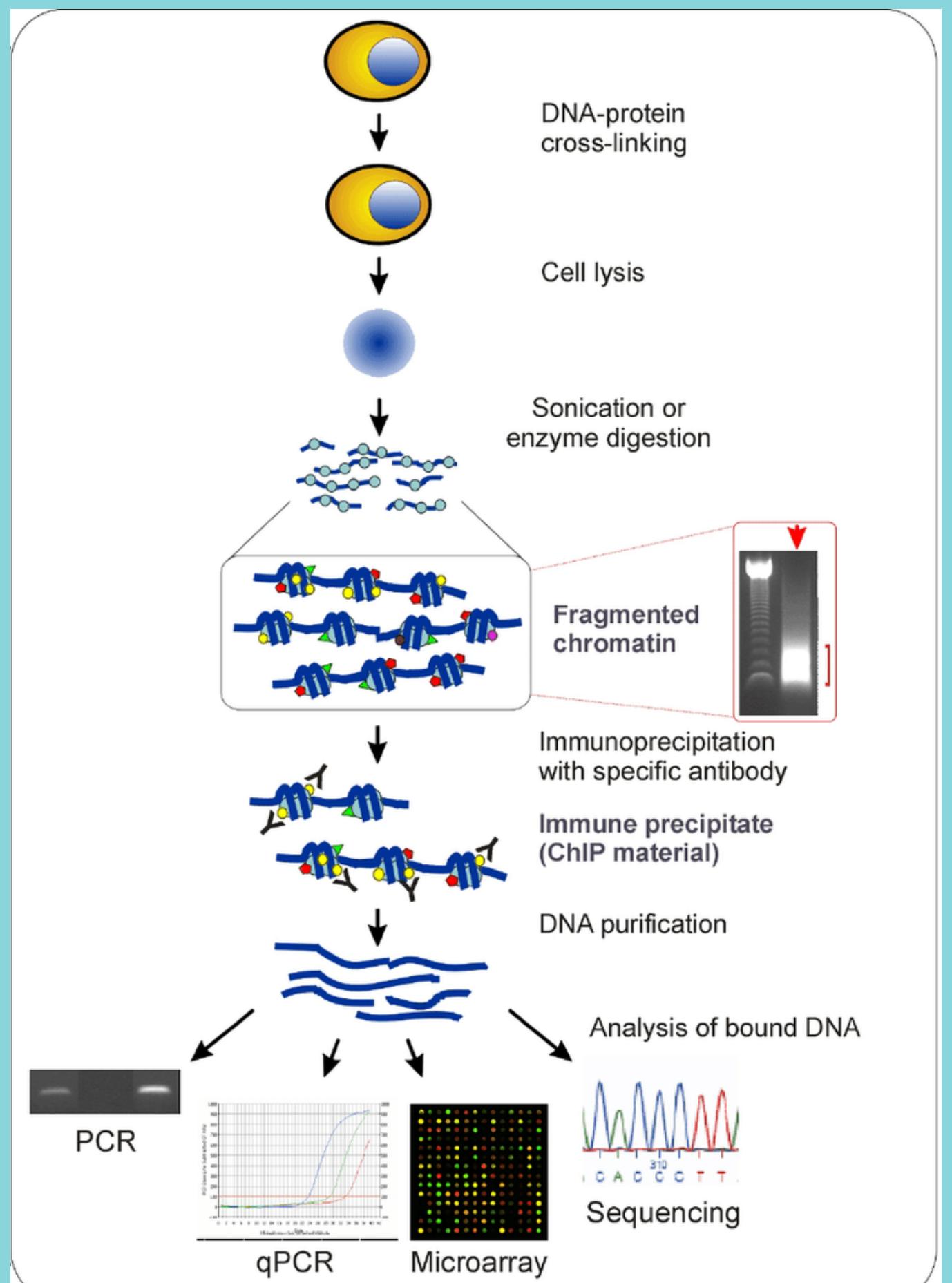


ChIP-Seq

ChIP-Seq (**chromatin immunoprecipitation sequencing**), which refers to the binding site analysis, is a way to analyze **DNA-protein interactions**. The technique combines chromatin immunoprecipitation (ChIP) with NGS to identify where the DNA binds to the associated proteins.

It can be used to pinpoint the **binding sites** of proteins across the genome. It reveals how transcription factors and chromatin-related proteins influence **phenotypic mechanisms**.

ChIP-Seq Workflow



Cross-linking

Cell Lysis

Chromatin Fragmentation

Immunoprecipitation

Reversal Cross Linking

DNA Analysis

Cross-linking: The process begins by treating cells with a cross-linking agent to fix protein-DNA interactions in place.

Chromatin Extraction: Cells are lysed to isolate chromatin, which consists of DNA wrapped around histone proteins and associated proteins. The chromatin is fragmented into smaller pieces .

Immunoprecipitation (IP): Antibodies specific to the protein of interest are used to selectively pull down the protein-DNA complexes from the chromatin mixture.

Washing: Weakly bound molecules are removed through a wash steps, leaving only the protein-DNA complexes bound to the antibodies.

Reverse Cross-linking: The cross-links between proteins and DNA are reversed, freeing the DNA fragments from the protein complexes.

DNA Purification: The DNA fragments are purified from the mixture.

Library Preparation: The purified DNA fragments are then prepared into a sequencing library. This involves adding adapter sequences to the DNA fragments.

Sequencing: The prepared library is sequenced using next-generation sequencing (NGS) technologies. This step generates millions of short DNA sequences, representing the DNA fragments from the original chromatin mixture.

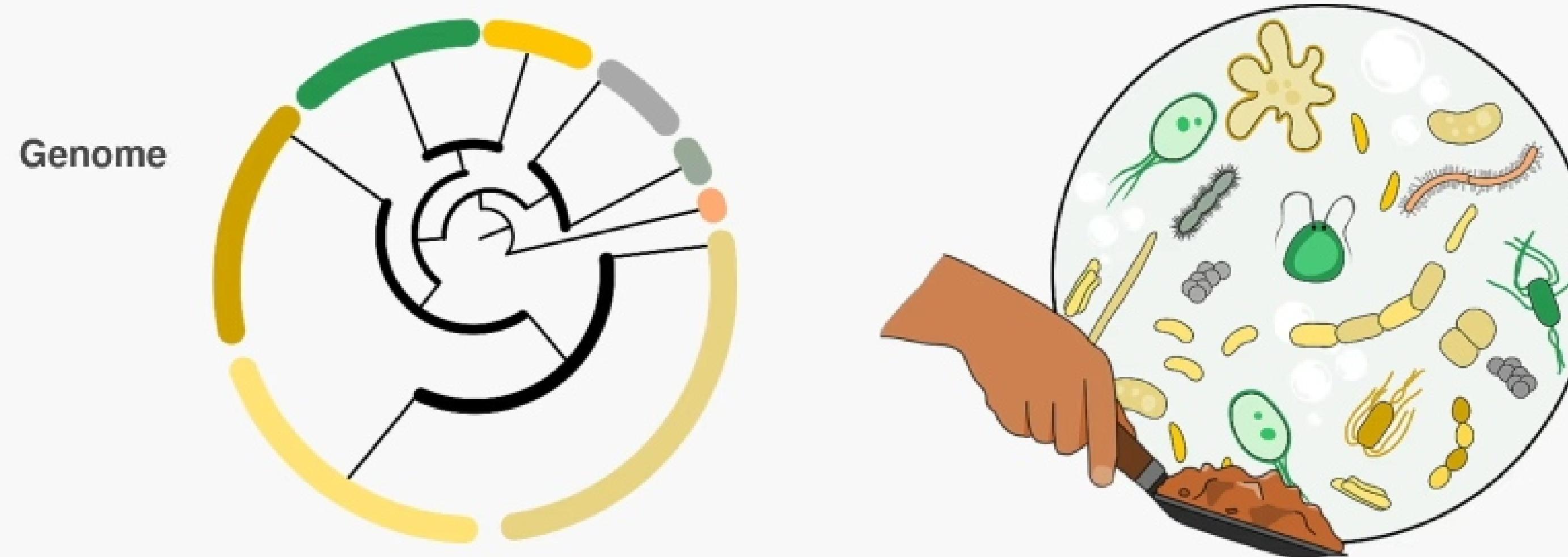
Application of ChIP-Seq:-

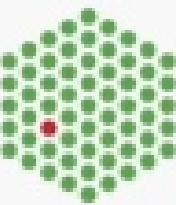
- Gene regulation
- Transcription complex assembly
- DNA repair
- Histone modification
- Developmental mechanisms
- Disease progression

Metagenomics

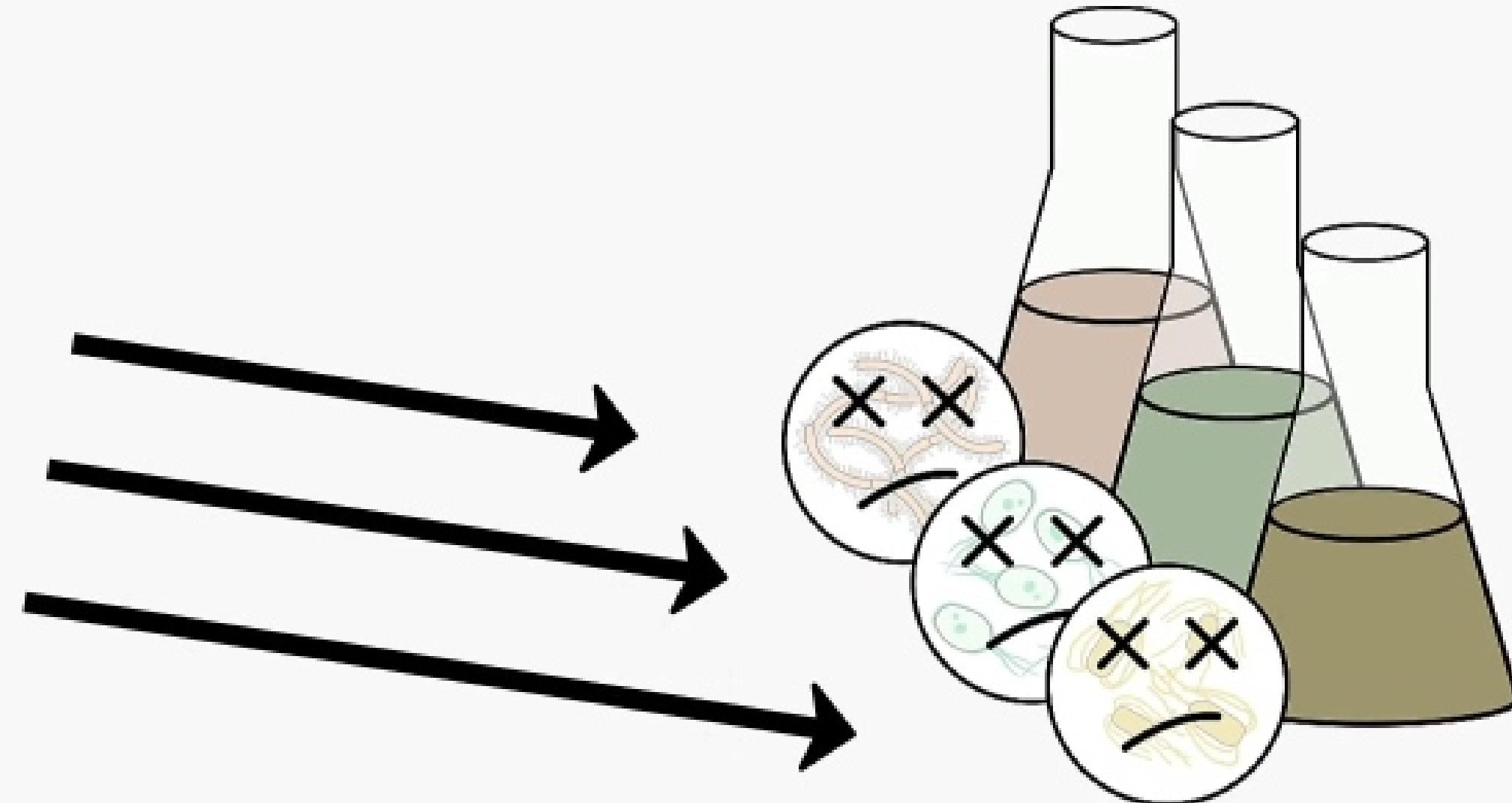
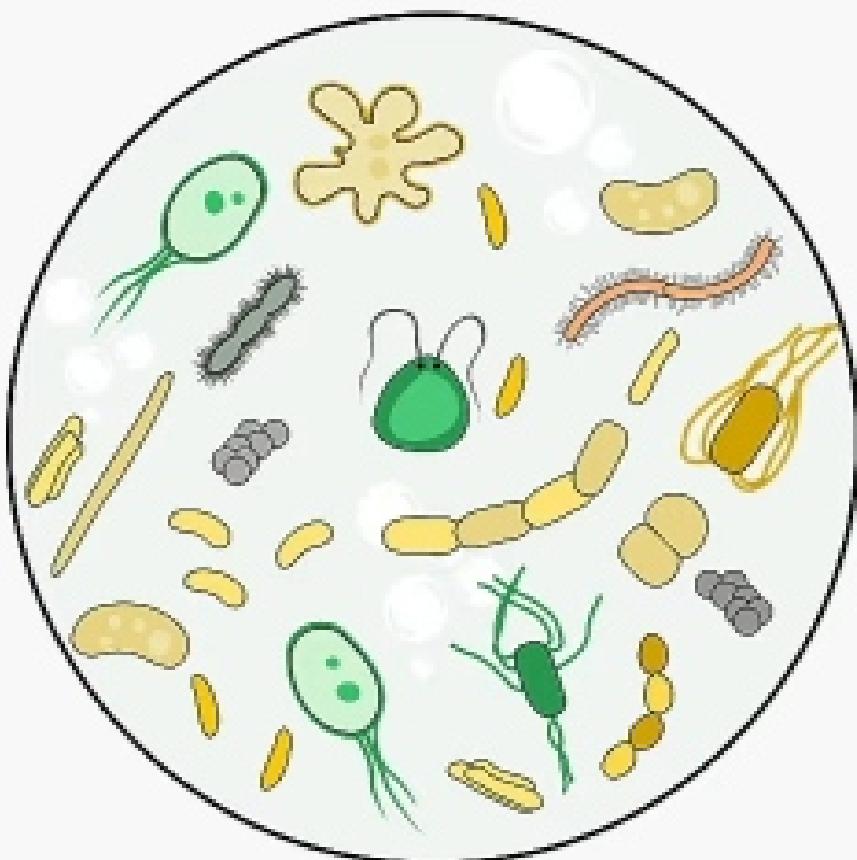
Metagenomics is the genomic analysis of microbial communities by extraction and sequencing their DNA , that allow us to studying communities of organism directly in their natural forms.

What is the Metagenome?

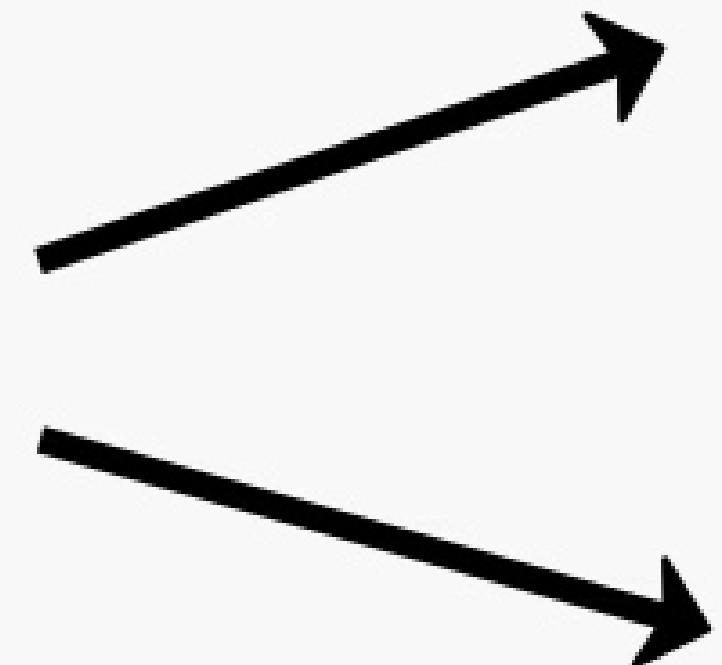
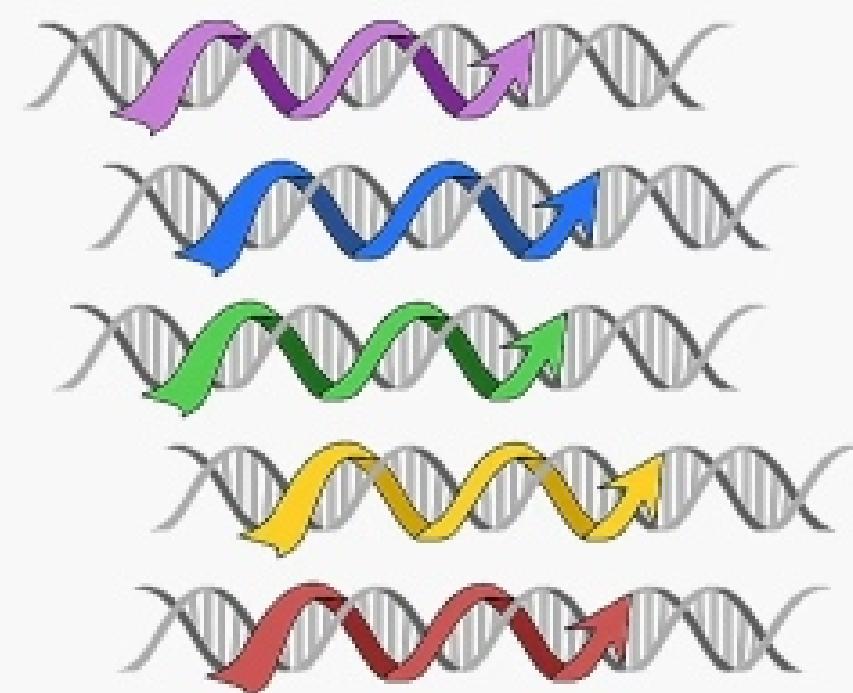
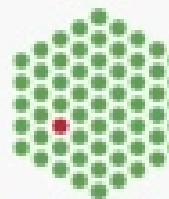




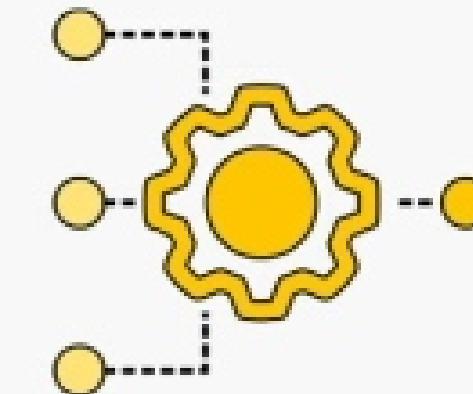
Why do we study the metagenome?



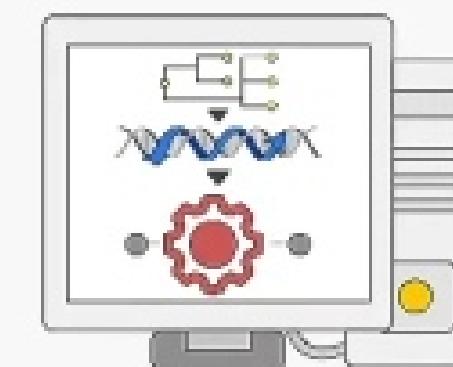
How to study the metagenome?



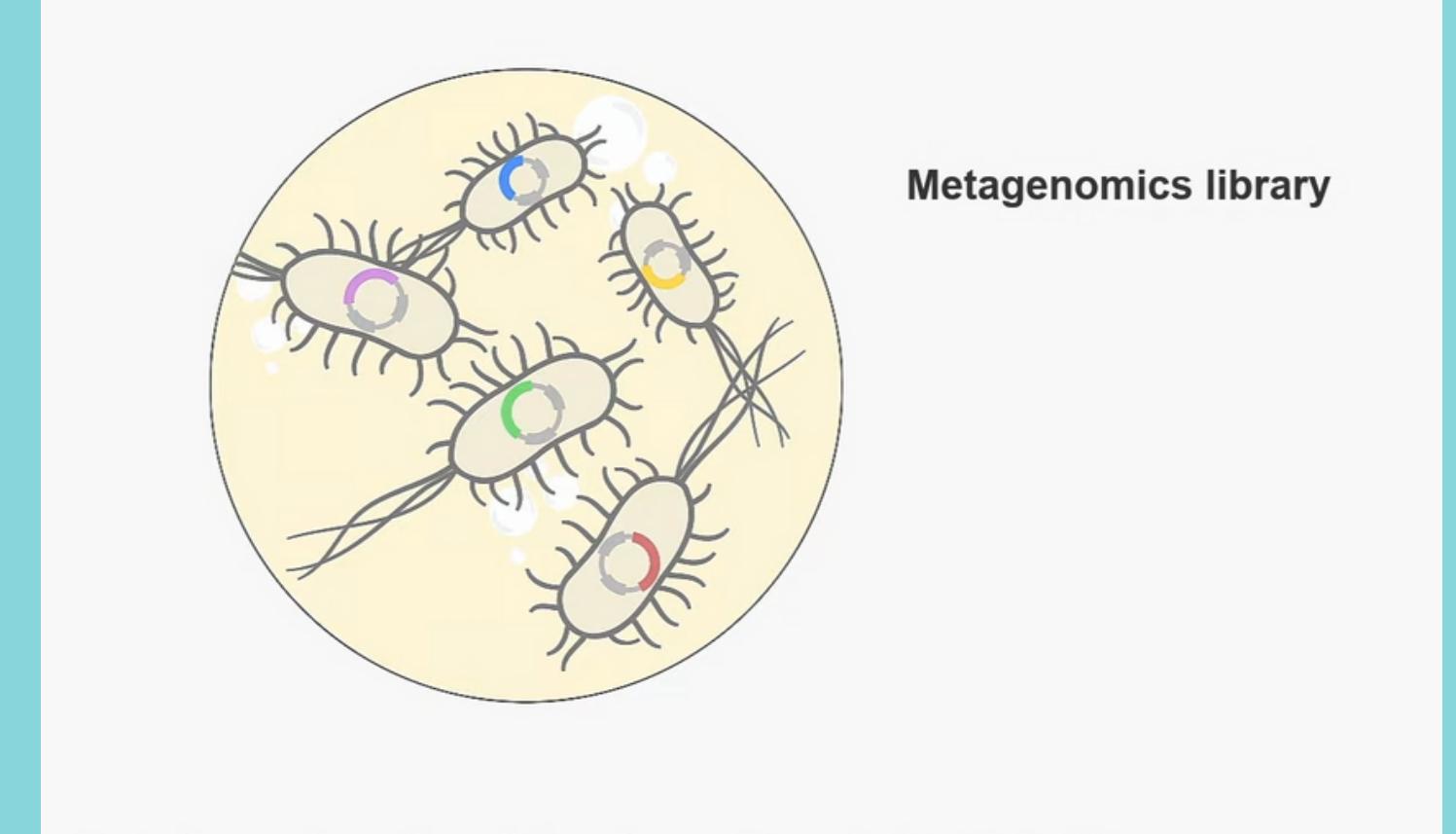
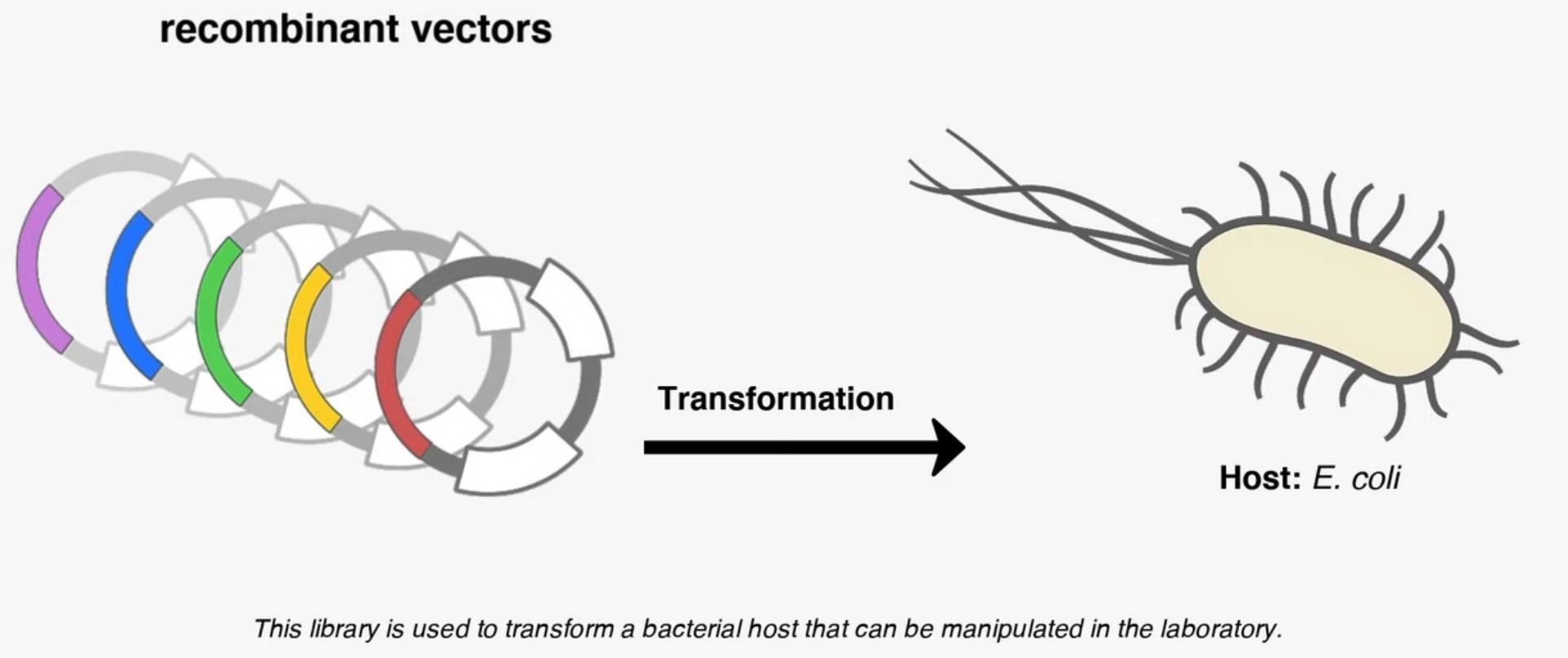
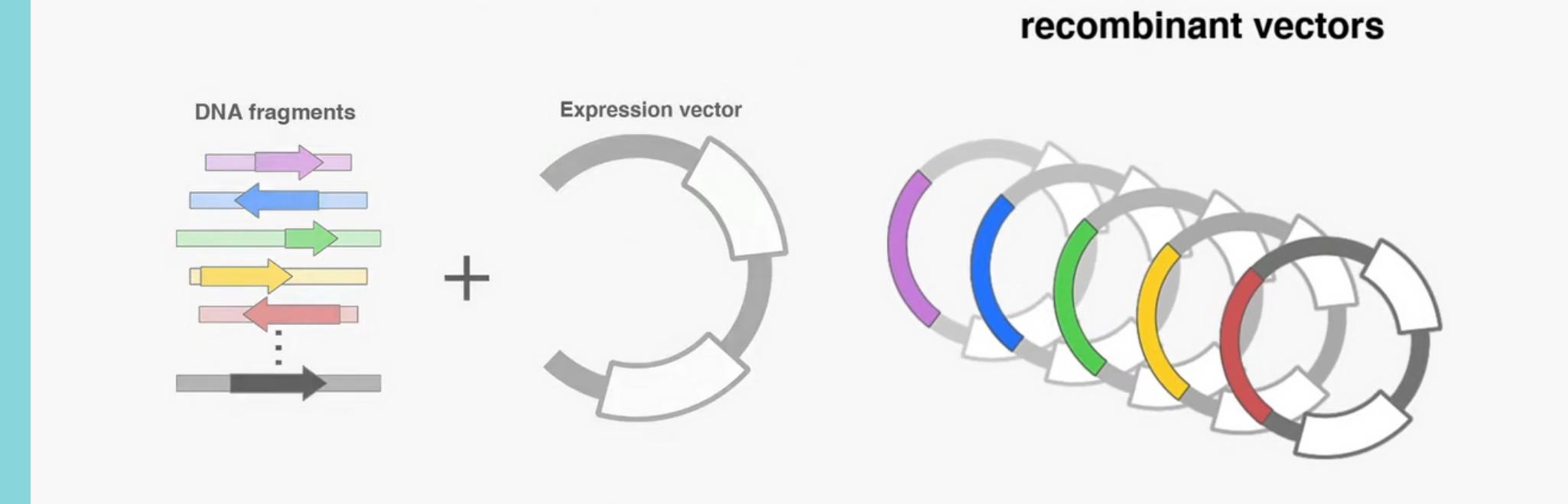
A) Functional Metagenomics



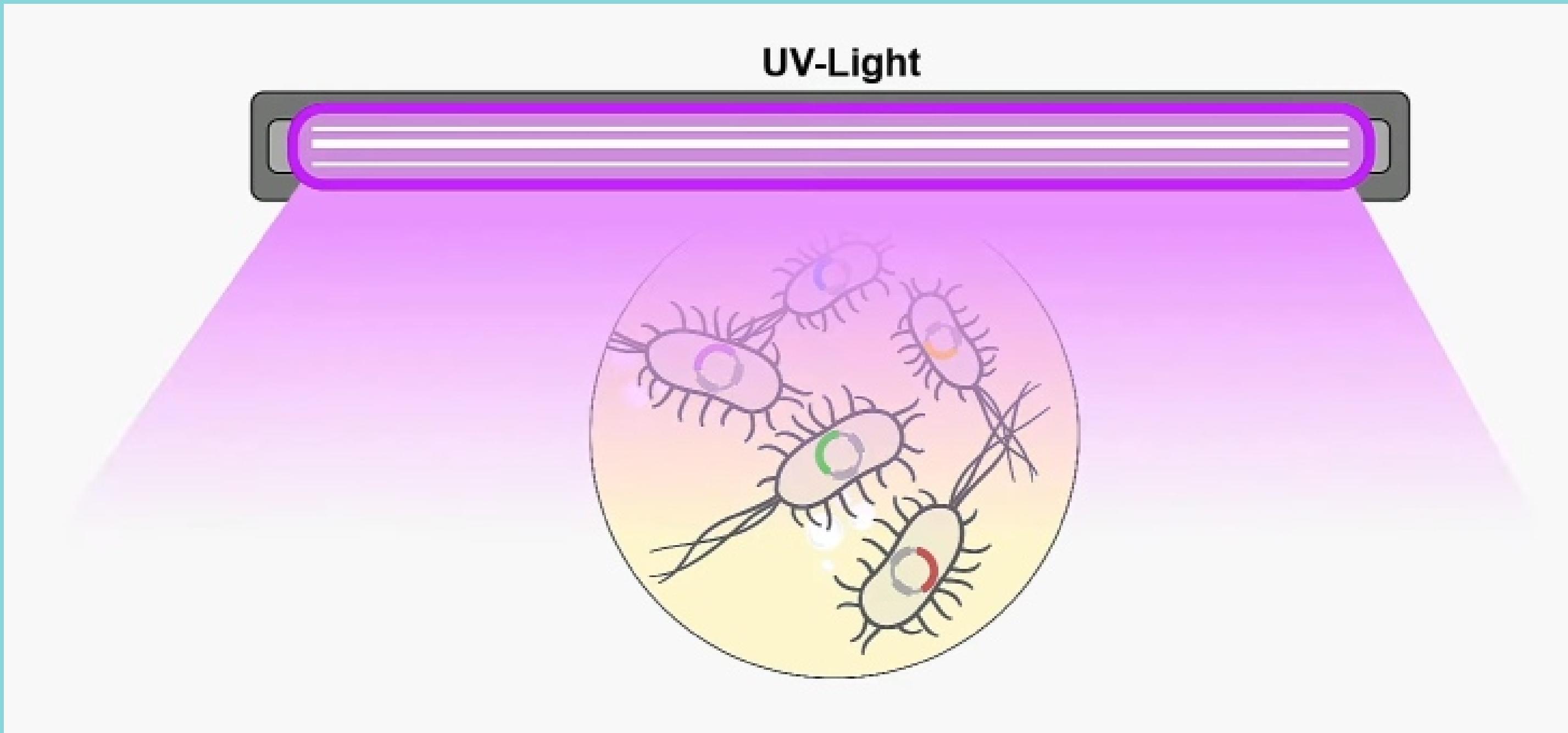
B) Metagenomics Sequencing



A. Functional metagenomics

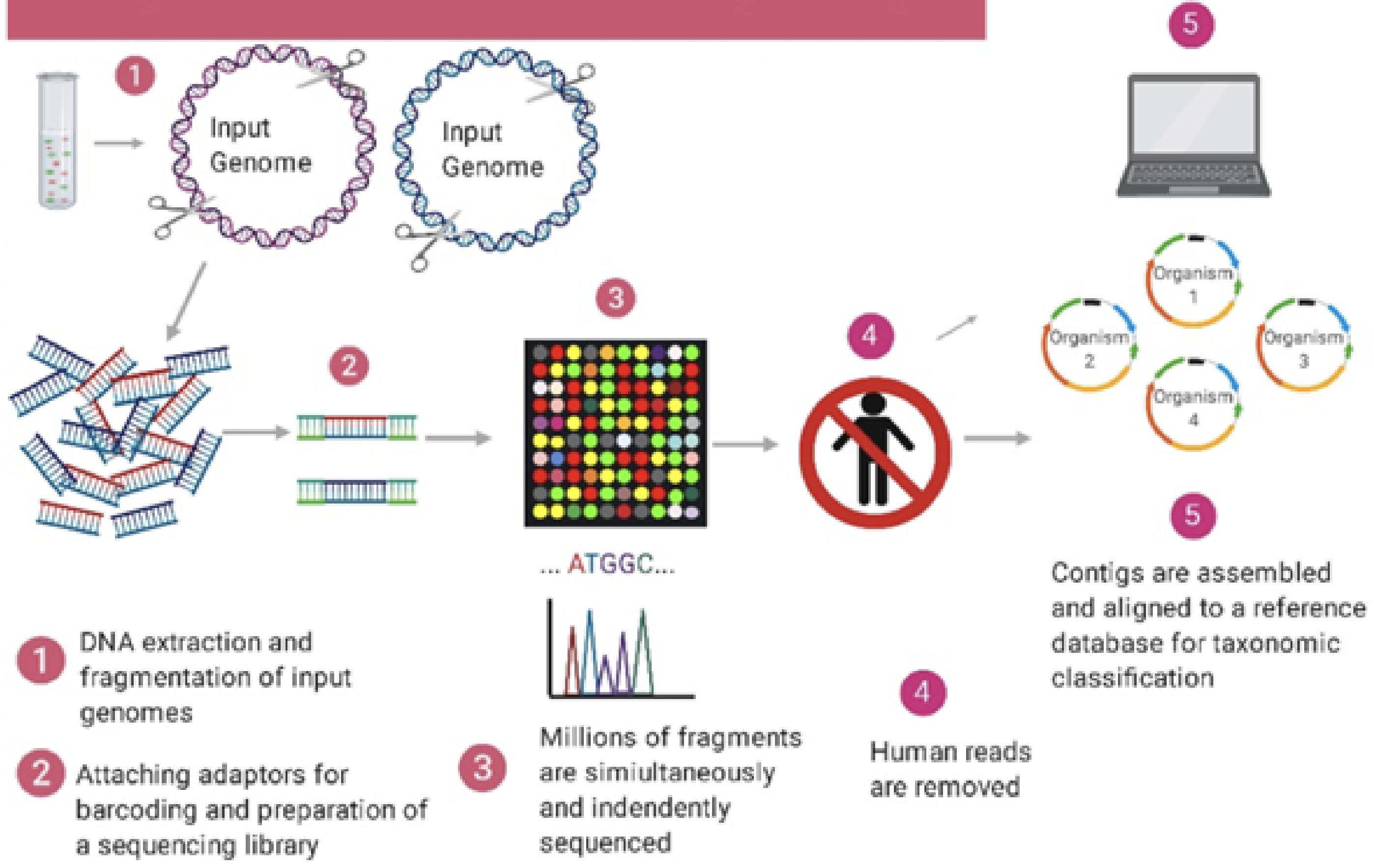


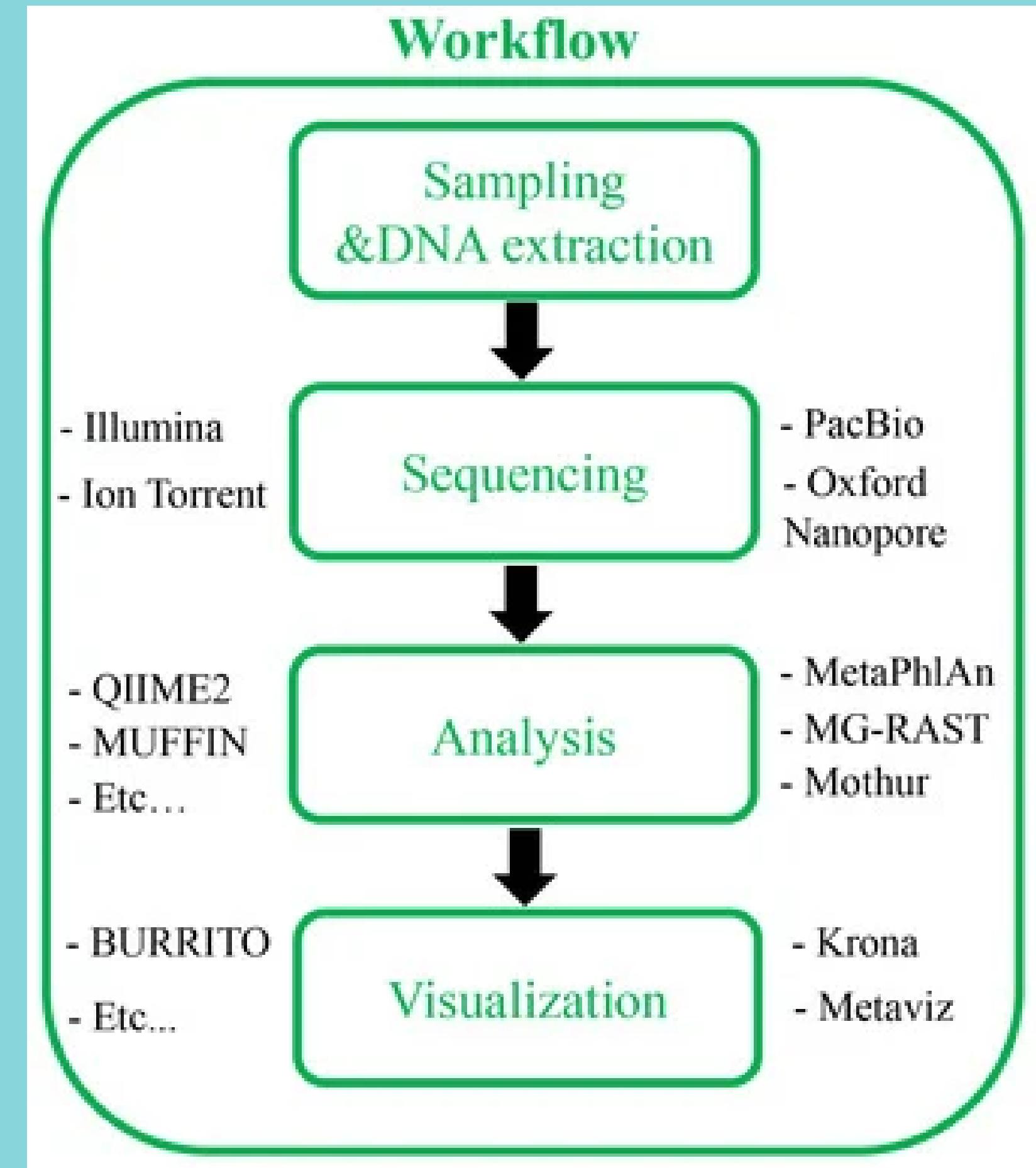
Example :



B.Metagenomics sequencing

Metagenomic Next-Generation Sequencing





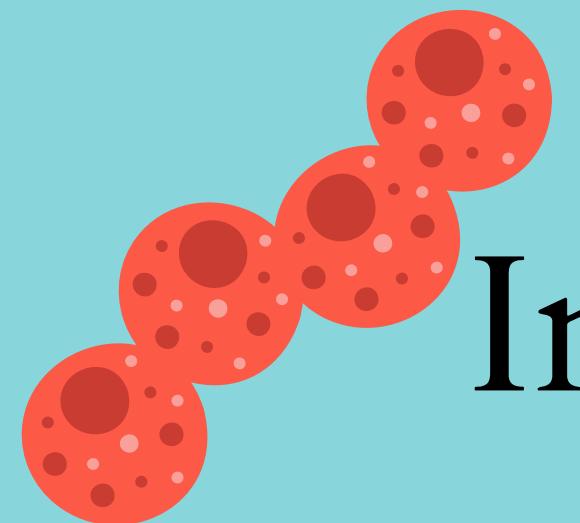
Advantages

- Unveils unseen microbial diversity
- Provides functional insights into microbial communities

Disadvantages

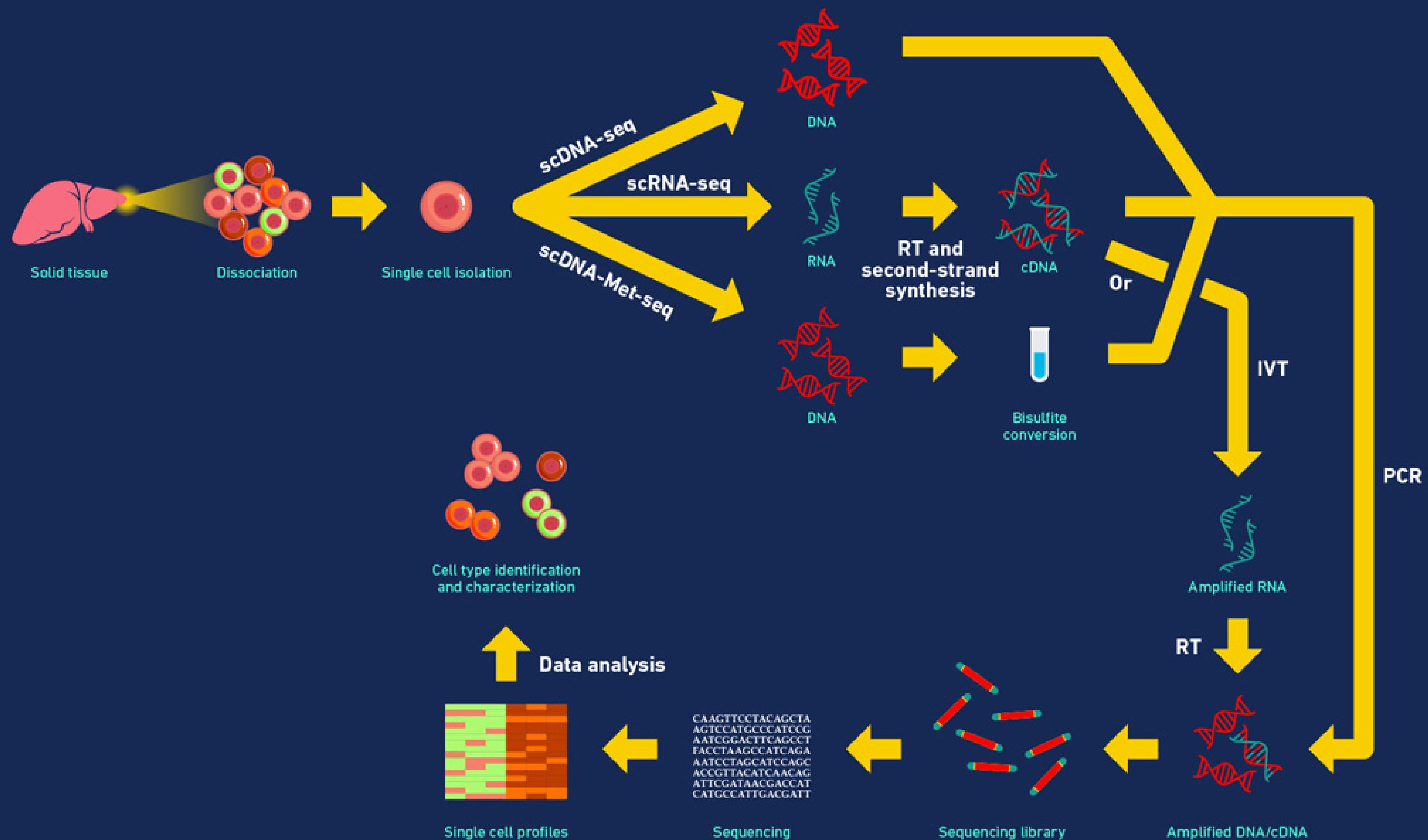
- Complex data analysis
- High cost of metagenomic studies

Single Cell Sequence Technology:



Introduction:

1. Single-cell sequencing technology = To analyze the genetic information of individual cells.
2. Traditional sequencing methods often require millions of cells, masking the diversity and heterogeneity present within a tissue or organism.
3. Single Cell Sequencing process typically involves several steps:
 - Isolation of single cells,
 - Amplification of their genetic material (DNA or RNA),
 - Sequencing
 - Data analysis.



Isolation of cells and basic sample processing:

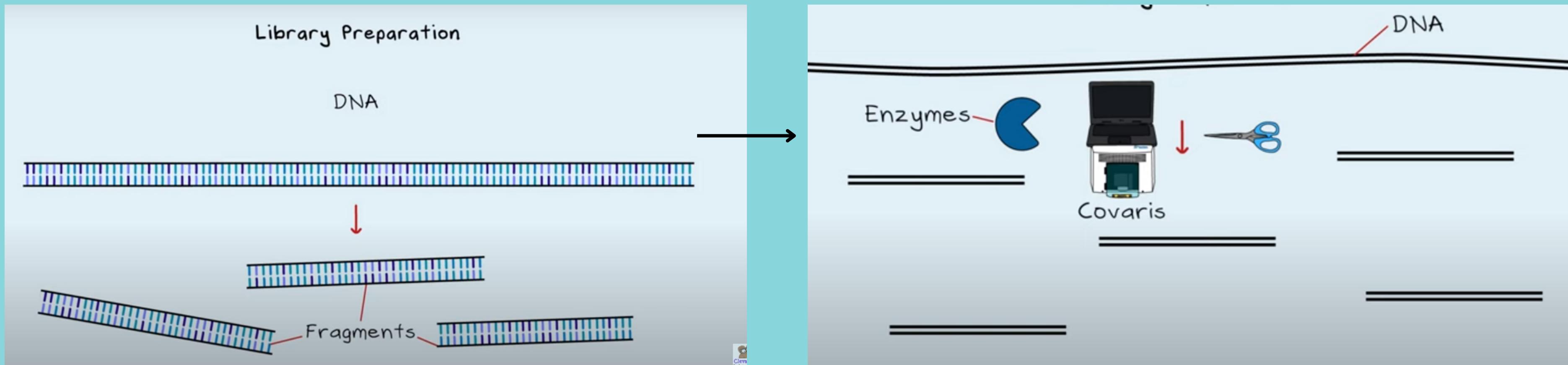
Cells can be isolated using different methods the choice of which mainly depends on the nature of the sample and the processing steps required after the cells' isolation.

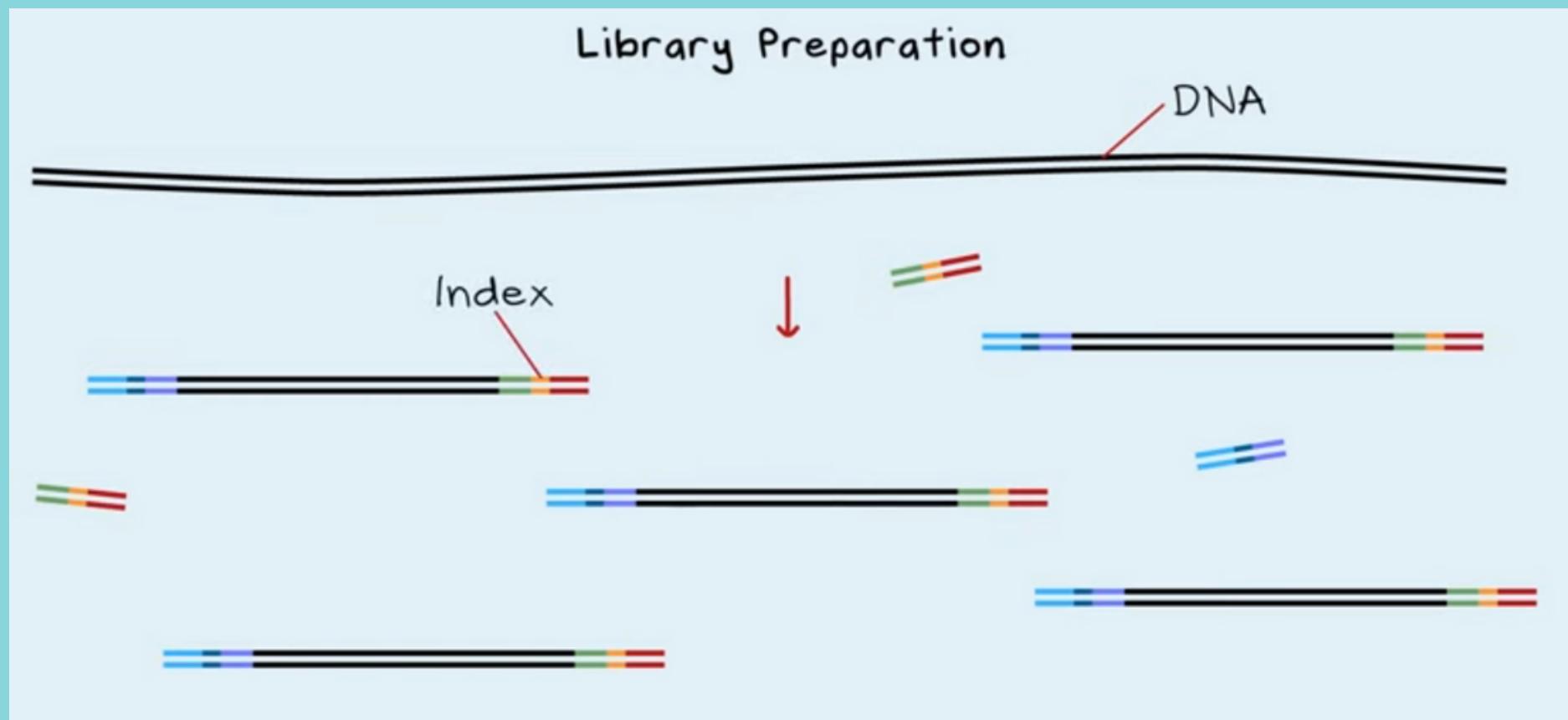
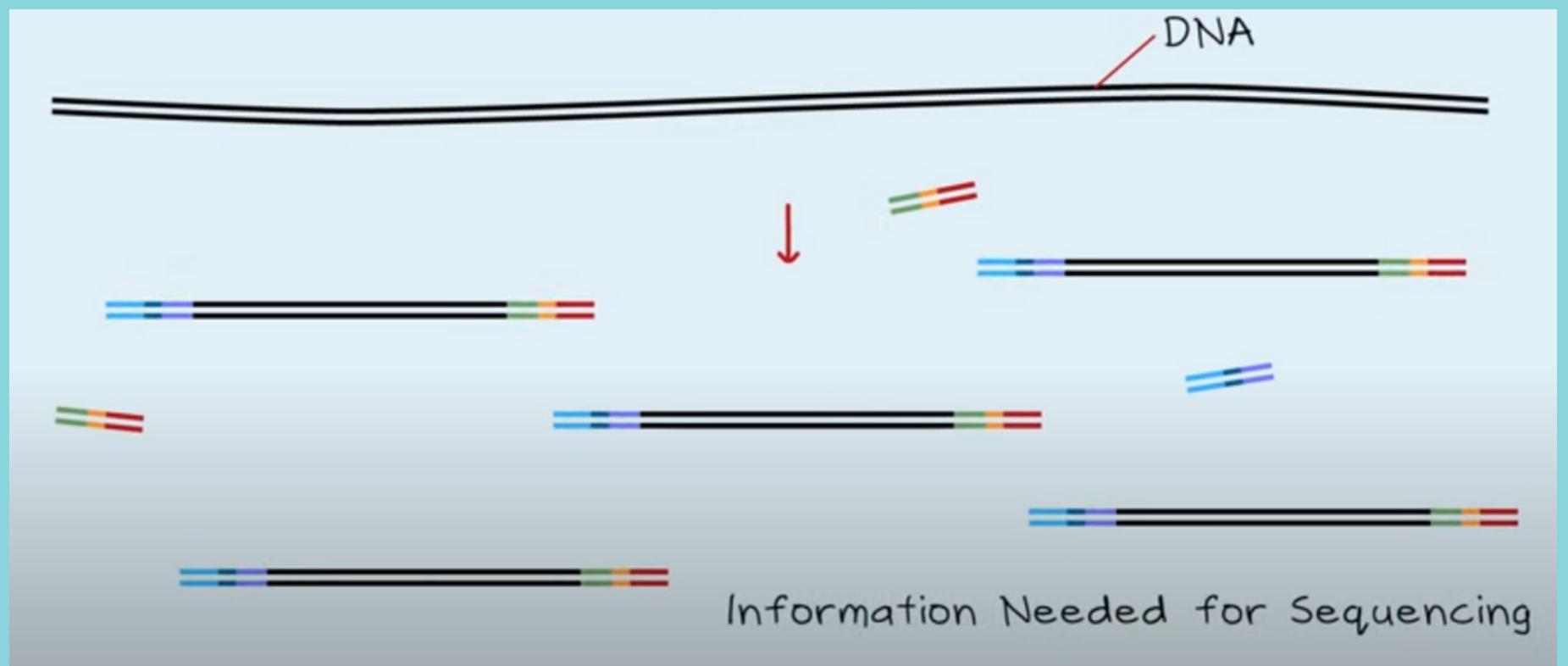
Methods:

- Fluorescence-activated cell sorting (FACS)
- Magnetic-activated cell sorting (MACS)
- Laser capture microdissection (LCM)
- Manual cell picking, or micromanipulation

Sequencing library preparation

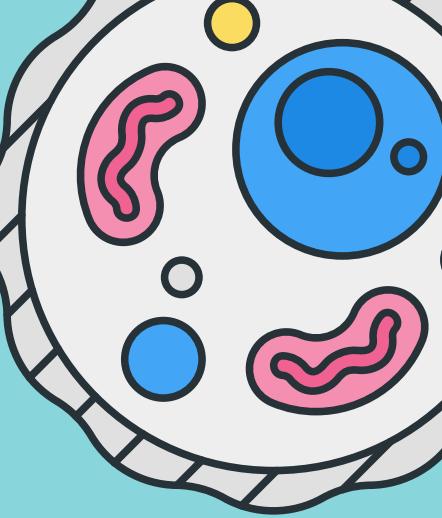
For the amplified DNA to be sequenced, it first needs to be made into a sequencing library.



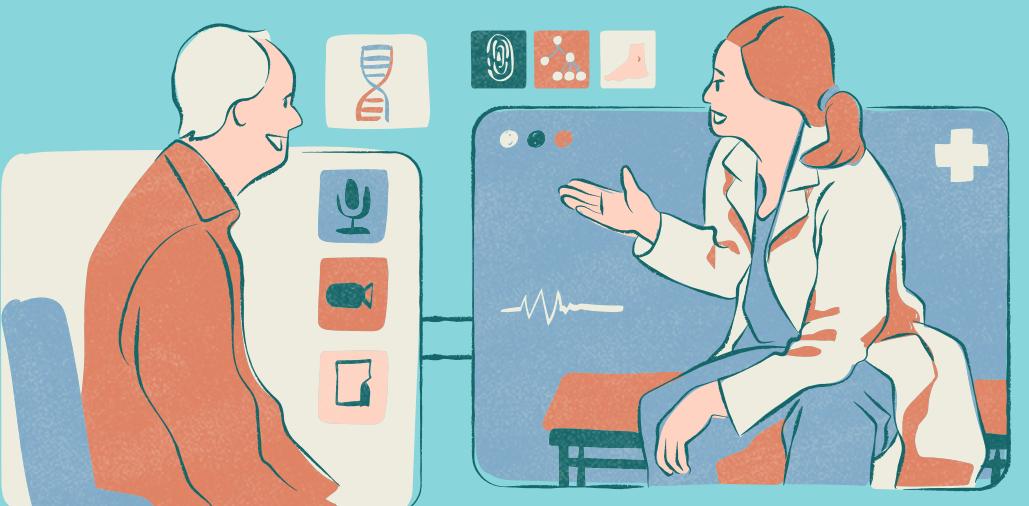




Analyzing single-cell sequencing data



- The final raw output of the sequencer is first processed directly within the sequencing machine, returning the binary base call (BCL) file and quality scores.
- The BCL file is the raw sequencing output in binary format.
- For further analysis, the BCL file is then converted into a FASTQ file, a text file containing the sequence information and quality scores .
- This step is usually performed on a Linux server after demultiplexing the data from different libraries using the barcode tags.





Why is single-cell sequencing important?

- With single-cell technologies, we can probe each cell and measure its specific contribution to the whole cell population – and its organism or ecosystem.
- Single-cell sequencing technology has revolutionized fields such as:
 1. Cancer research,
 2. Immunology,
 3. Neurobiology,
 4. Developmental biology.



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